6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring diazinon, its metabolites, and other biomarkers of exposure and effect to diazinon. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

In the design of a study and the selection of an analytical method, it is very important that adequate attention be paid to the extent of validation and field applicability of a particular method. Not all of the methods have been validated to the same extent. It is the analyst's responsibility to determine the data quality needed before initiating the application of a particular method.

The analytical methods used to quantify diazinon in biological and environmental samples are summarized below. Table 6-l lists the applicable analytical methods for determining diazinon in biological fluids and tissues and Table 6-2 lists the methods used for determining diazinon in environmental samples.

6.1 BIOLOGICAL SAMPLES

Diazinon is widely used for agricultural purposes, and residues on or in foods can result in exposure of humans by ingestion. Additional exposure potentials exist as a result of home gardening activities. Consequently, methods for the determination of diazinon in biological samples can be used to verify that exposure and absorption have occurred. Since diazinon is rapidly metabolized, determination of the parent compound can provide evidence only of very recent exposures (see Chapter 2). Methods have been reported for metabolites, and these are discussed below under Biomarkers of Exposure.
A few papers were found that deal with the determination of diazinon in human samples and these are described below. Some methods have reported the determination of diazinon in animal tissue or other

Table 6-1. Analytical Methods for Determining Diazinon and Transformation Products in Biological Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human fatty tissue (from greater omentum)	Tissue pulverization and extraction with acetone. Concentration and purification by sweep co-distillation and Florisil/anhydrous sodium sulfate column chromatography. Elution with 20% ether in hexane followed by hexane. Addition of internal standard.	GC/NPD	No data	No data	Kirkbride 1987
Human adipose, bile, blood, brain, stomach contents, kidney, and liver	Maceration of 0.5 g sample in tissue grinder with acetonitrile. Addition of aqueous sodium sulfate and partitioning into hexane. Concentration and clean up using Florisil column.	GC/ECD; GC/FID	No data	No data	Poklis et al. 1980
Human urine (DEP, DETP)	Dilution of urine with acetonitrile, azeotropic distillation for water removal, evaporation of solvent, redissolution in acetone and derivatization using pentafluorobenzyl bromide.	GC/FPD	DEP: 0.072 ppm; DETP: 0.041 ppm	DEP: 96 (4.7% RSD); DETP: 99 (2.4% RSD) at 0.8 ppm.	Reid and Watts 1981
Dog urine (2-isopropyl-4-methyl-6-hydroxypyrimidine; 2-1'-hydroxy-1'-methyl)-ethyl-4-methyl-6-hydroxypyrimidine)	Extraction with chloroform, volume reduction, alkylation or silylation	GC/electrolytic conductivity detection	< 1 ppm	No data	Lawrence and Iverson 1975

Table 6-1. Analytical Methods for Determining Diazinon and Transformation Products in Biological Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bovine liver, rumen content (partially digested grain and vegetation mixture)	Extraction of homogenized sample with methanol-dichloromethane (10–90, v/v) followed by gel permeation chromatography and silica gel solid phase extraction clean-up.	GC/FPD	0.01–0.05 µ/g using 5 g sample	Rumen content: 95 (3% RSD) at 0.1 µg/g; liver: 88 (5% RSD) at 0.05 µg/g	Holstege et al. 1991
Avian liver, kidney	Homogenization with HCl/ethanol/ethyl acetate, centrifugation and evaporation of supernatant to dryness. Re-dissolution in hexane, filtration and clean up using GPC and volume reduction.	GC/FPD; GC/MS	0.02 ppm	100 (3% RSD) from liver at 0.5 µg/g (0.5 ppm)	Richardson and Sieber 1993
Animal fat	Sweep codistillation, Florisil clean up-elution with methylene chloride-light petroleum-acetonitrite (50+48.5+1.5)	GC/FPD	No data	90 (6% RSD) at 0.4 mg/kg	Brown et al. 1987

[a] Diazinon is the target analytes unless otherwise specified.

DEP = O,O-Diethyl phosphate; DETP = O,O-Diethyl phosphorothionate; ECD = electron capture detector; FPD = flame photometric detector; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry; NPD = nitrogen phosphorus detector

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air, gloves (surrogate for dermal exposure)	Preconcentration from air sample using polyurethane foam (PUF). Soxhlet extraction of PUF or gloves with 5% ethyl ether/hexane. Addition of deuterated internal standards and concentration using K-D and nitrogen blowdown.	Capillary GC/MS (can use multiple ion detection)	55 ng/m^3 (5.5 m^3 sample)	73 (14% RSD)	Hsu et al. 1988
Air (diazinon, diazoxon)	Preconcentration using ORBO-42 pesticide adsorbent tubes (Supelco). Extraction with acetone, evaporation just to dryness and redissolution in 100 µL acetone containing internal standard.	Capillary GC/NPD	No data	≥90 at 0.1 and 1 µg/m^3 (diazinon)	Williams et al. 1987
Air	Preconcentration of pesticide onto OVS-2 tube (13 mm quartz filter, XAD-2, 270 mg/140 mg. Elution with 90% toluene/10% acetone.	GC/FPD (NIOSH Method 5600)	0.0004 mg/m^3 (400 ng/m^3) for 120 L sample.	94 (2.7% RSD at 2.4 µg (0.01 µg/m^3, 240 L sample)	NIOSH 1994
Air	Preconcentration of diazinon from air onto activated carbon fiber filter. Elution with benzene:ethanol (4:1 volume:volume) followed by volume reduction.	GC/MS	0.5 ng/m^3	95 ± 4.7% at 10 L/min sampling flow, 31 °C and 85 relative humidity	Kawata and Yasuhara 1994
Drinking water	Preconcentration onto 5 µm C_{18}-silica or 7 µm polystyrene-divinyl benzene co-polymer with subsequent backflush onto analytical HPLC column.	RP-HPLC/UV (254 mn)	0.03–0.06 µg/L (ppb)	91 (±10% RSD) at sample volumes up to 300 mL	Driss et al. 1993

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, river water	Preconcentration of 2.5 mL water onto C_{18} extraction disks, rinsing with additional 1 mL and purging disk with gas to remove residual water. Elution with ethyl acetate directly onto GC pre-column with solvent venting.	GC/NPD	Tap water: 20 pg/mL (ppt); river water: 20–50 pg/mL	>95 (<4% RSD at 200 ppt)	Kwakman et al. 1992
Pond water	Micro liquid-liquid extraction of 1.5 mL water with 1.5 mL methyl t-butyl ether. 500 µL of extract slowly introduced into GC pre-column with solvent venting.	cap. GC/FPD	0.02 µg/L (ppb)	102 (5% RSD) at 0.50 µg/l level	van der Hoff et al. 1993
Surface water	Adsorption of pesticides from 2 L of water onto XAD-2 and XAD-7 resins. Elution with methylene chloride, water removal and use of K-D to reduce volume.	GC/chemical ionization ion trap MS	0.0005 ppb (0.5 ppt)	103.8 (14% CV) at 1 ppb level	Mattern et al. 1991
Water	1. Liquid/liquid extraction (EPA Method 3510); 2. continuous liquid/liquid extraction (EPA Method 3520).	GC/FPD (EPA Method 8140)	6 µg/L (ppb)	No data	EPA 1986a, 1986c, 1986d
Water	Filtration of 1 L of water followed by extraction 3 times with 100 mL methylene chloride after addition of 20 g sodium sulfate. Concentration using K-D and solvent exchange to benzene. Concentrations done under nitrogen. Fractionation by HPLC.	GC/FPD (P-mode)	0.025 µg/kg (ppb or 25 ng/kg, ppt)	92 (2% RSD)	Seiber et al. 1990

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Solid-phase microextraction (SPME) of filtered water sample; thermal desorption of diazinon from SPME fiber.	GC/AED	1 µg/L (ppb) with carbon line (193 nm); 3 µg/L with S line (181 nm).	No data (precision 8–12 relative standard deviation)	Eisert et al. 1994
Water	Extraction of analytes from water using SPE; elution with ethyl acetate (108 µL) directly onto retention gap with solvent venting.	GC/AED	1 ng/L (100 mL sample) with P channel.	105 (4% RSD) at 5 µg/L.	Hankemeier et al. 1995
Industrial and municipal waste water	Extraction of 1 L of sample with 60 mL methylene chloride 3 times. Water removal from extract and solvent exchange to hexane during K-D concentration.	GC/FPD or thermionic detection (P-mode); GC/MS for qualitative identifications recommended. (Method 1657)	0.6 µg/L (ppb)	67 (6% (RSD)	EPA 1992a
Waste water	Extraction of 1 L of water with 15% methylene chloride in hexane using a separatory funnel. Concentration using K-D. Clean up (if needed) by Florisil fractionation or acetonitrile partition.	GC/FPD (P-mode) or GC/thermionic detection. GC/MS for qualitative compound identification recommended. (Method 614)	0.012 µg/L (ppb); (12 ng/L, ppt)	94 (5.2% RSD)	EPA 1992b

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Direct injection or liquid/liquid extraction and concentration.	HPLC/UV	0.5 mg/L (ppm, direct injection); 0.5 µg/L (ppb, liquid/liquid extraction)	No data	Mallet et al. 1990
Soil (diazinon, diazoxon, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine)	Extraction of soil with hexane:acetone (1:1), centrifugation, separation of hexane from acetone/water layer. Extraction of acetone/water phase with chloroform:diethyl ether (1:1), solvent exchanged to methanol. Hexane layer contained diazinon, chloroform/diethyl ether fraction contained 2-isopropyl-4-methyl-6-hydroxy-pyrimidine.	TLC	No data	No data	Sethunathan and Yoshida 1969
Soil (diazinon, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine)	Sequential Soxhlet using acetone then methanol.	GC, TLC, GC/MS	No data	No data	Burkhard and Guth 1979

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, soil	Water: Addition of deuterated standards to 1 L water and extraction 3 times with 200 mL methylene chloride. Water removal with anhydrous sodium sulfate then concentration using K-D and nitrogen blowdown. Soil: Addition of 10 mL water and deuterated standards to 50 g of soil followed by equilibration for 1 h. Sonication 3 times with acetone/hexane. Phase separation followed by water removal using sodium sulfate, concentration using K-D, and nitrogen blow-down. Spiking with phenanthrene-d_{10} before analysis.	GC/MS(SIM)	100–200 ppt for water, 2–4 ppb for soil	Water: 89.4 (4.4% RSD) at 1 ppb Soil: 103 (15% RSD) at 20 ppb	Lopez-Avila et al. 1985
Waters, soils, sediments, sludges	<30% solids: Dilution to 1% solids and extraction with methylene chloride, concentration using K-D. Clean up using GPC and SPE. >30% solids: Sonication with acetonitrile and methylene chloride; back extraction with water, concentration using K-D; clean up using GPC and SPE.	GC/FPD (Method 622)	3.8 ng/L	60–120	EPA 1992c

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soils, sludges, and solid waste	1. Soxhlet extraction (EPA Method 3540). 2. Sonication (EPA Method 3550). Cleanup using Florisil (EPA Method 3620) or GPC (EPA Method 3640).	GC/FPD (EPA Method 8140)	Low-level soil by sonication/ GPC cleanup: 400 µg/L; High-level soils and sludges by sonication: 6 mg/L; Non-water miscible waste: 60 mg/L	No data (depends on matrix)	EPA 1986a, 1986e, 1986f, 1986h, 1986i
Cucumber, lettuce, grapes	Chopping of produce and extraction with acetone/methylene chloride/petroleum ether (1:1:1). Evaporation to dryness and redissolution in acetone and concentration.	SFC/NPD	No data	No data	Zegers et al. 1994a
Green beans, lettuce, carrot, bell pepper	Homogenization of produce with acetonitrile. Addition of NaCl to affect phase separation, removal of acetonitrile, water removal volume reduction, addition of deuterated internal standards.	GC/MS	50 ppb	88 (17% RSD)	Liao et al. 1991
Kale, endive, carrots, lettuce, apples, potatoes, strawberries	Extraction of crops with ethyl acetate and granular sodium sulfate, filtration, concentration with K-D. Sweep co-distillation cleanup for GC. Florisil partition chromatography for polarographic confirmation.	GC/KCl thermionic detector or GC/FPD; polarographic confirmatory method	No data for GC; polarographic: 0.2 ppm based on 1 g crop in 1 mL cell	No data	AOAC 1990

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Numerous non-fatty crops	Extraction with acetonitrile and partition into petroleum ether. Concentration using K-D and purification using Florisil column chromatography.	GC/KCl thermionic detector; identifications by combinations of gas, thin layer, and paper chromatography	No data	≥80	AOAC 1990
Soybeans and rice	Grinding of 25 g samples and extraction with 150 mL of 2:1 acetone: methanol; filtration and reduction of volume to 100 mL. Addition of water, NaCl followed by extraction with methylene chloride (2x);solvent evaporation and redissolution in methylene chloride:cyclohexane (1:1) and fractionation on Bio-Bead S-X3. Evaporation under N_2 stream and redissolution in 2 mL hexane.	GC/NPD or GC/MS (SIM)	Rice: 0.01 ppm soybeans: 0.05 ppm	Rice: 83.4 (1.5% RSD) at 1 ppm soybeans: 62.7 (8.6% RSD) at 1 ppm	Hong et al. 1993
Various fruits and vegetables	Homogenization of sample (adding water if needed) and adsorption on activated Florisil to produce a free-flowing powder. Elution with ethyl acetate or methylene chloride.	GC/NPD	4 µg/kg (ppb)	91–103 at 0.05 mg/kg	Kadenczki et al. 1992
Various produce	Homogenization of sample and extraction with acetonitrile, filtration, addition of salt and solvent evaporation. Redissolution of residue in acetone for analysis.	GC/FPD or alkali FID	100 ppb	96 (17% RSD)	Hsu et al. 1991

Table 6-2. Analytical Methods for Determining Diazinon and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Various prepared foods	Blending of sample with acetone, filtration and transfer to Hydromatrix column. Elution with methylene chloride and concentration.	GC/FPD	No data	91 at 100 ppb	Hopper 1988
Pasta, eggs	Blending of samples with acetone and extraction with dichloromethane and acetone, water removal and volume reduction. Cleanup using carbon-celite (pasta) or C_{18} SPE (eggs).	GC/FPD	approx. 1 ppb	Pasta: 80 at 30 ppb; eggs: 93 at 13 ppb	Leoni et al. 1992
Milk	Extraction of milk 3 times with 70% acetonitrile in water, filtration, removal of fat by zinc acetate addition, and partitioning with methylene chloride. Reduction of volume after drying.	GC/FPD (P-mode)	10 ppb	89 (3.8% RSD) at 100 ppb	Toyoda et al. 1990
Lanolin	Dissolution in hexane and extraction with acetonitrile. Addition of 5% NaCl in water to acetonitrile and back-extraction with hexane. Washing of hexane extract with water, volume reduction and fractionation using Florisil.	GC/FPD (526 nm); GC/atomic emission detection; GC/MS	GC/FPD 0.03 ppm; GC/AED 0.6 ppm(P); 0.3 ppm(S); GC/MS 0.6 ppm	90 (6.4% RSD) at 1 ppm 95 (5.6% RSD) at 2 ppm	Miyahara et al. 1992

[a] Unless otherwise stated, diazinon was determined

FPD = flame photometric detector; GC = gas chromatography; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; K-D = Kuderna-Danish; MS = mass spectrometry; NPD = nitrogen phosphorus detector; SFC = supercritical fluid chromatography; SIM = selected ion monitoring; SPE = solid phase extraction; UV = ultraviolet absorbance detection

6. ANALYTICAL METHODS

animal samples and should be applicable to human samples, but such an application would need to be validated.

Kirkbride (1987) described the estimation of diazinon in human omental tissue (fatty tissue) after a fatal poisoning. In this method, the tissue was pulverized and extracted with acetone. After extract concentration and purification by sweep co-distillation and Florisil fractionation, diazinon was measured by gas chromatography (GC) with nitrogen-phosphorus detection (NPD). After another fatal diazinon poisoning, diazinon was quantified by GC/electron capture detection (ECD) and GC/flame ionization detection (FID) by Poklis et al. (1980). The diazinon in human adipose, bile, blood, brain, stomach contents, kidney, and liver was recovered by macerating the sample with acetonitrile followed by the addition of aqueous sodium sulfate and extraction into hexane. Following an adsorption chromatography clean-up, the sample was analyzed.

A method for the determination of diazinon in human serum has recently been published by researchers at the Centers for Disease Control and Prevention (Liu et al. 1994) in which 2-dimensional chromatography was used to determine 15 pesticides in 4 minutes. Supercritical fluid extraction (SFE) was used to recover pesticides into methylene chloride and this extract was analyzed using two 2-meter columns connected by an on-column thermal desorption modulator. Sensitivity for diazinon was reported to be 1.8 pg on-column; no details about overall recoveries were provided.

Diazinon was determined in bovine liver and rumen content by GC/flame photometric detection (FPD) by Holstege et al. (1991) using a method with a limit of detection (LOD) reported to be 0.01-0.05 μg/g using a 5 g sample. Recoveries were reported to be 95% from rumen content and 88% from liver. In another study, diazinon was determined by GC/FPD and GC/mass spectrometry (MS) in avian liver and kidney using a method with a LOD of 0.02 ppm and 100% recovery at the 0.05 ppm level. Brown et al. (1987) used GUFPD to determine diazinon in animal fat. No data were reported for the LOD, but the recovery was stated to be 90% (6% RSD) at 0.4 ppm.

Animal fat was studied using sweep codistillation (Brown et al. 1987). Good recovery (90%) was measured at 0.4 mg/kg; no LOD information was given. Diazinon in liver and rumen content was determined by GC/FPD after methanoVdichloromethane (1:9) extraction and clean-up using either gel permeation chromatography (GPC) or silica gel solid phase extraction (SPE). The LODs were

reported to range from 10 to 50 μg/g using a 5 g sample with measured recoveries of diazinon from rumen content of 95% at 0.1 μg/g and from liver of 88% at 0.05 μg/g.

The mode of injection in GC-based methods can affect the recoveries of diazinon. In a study of the determination of organophosphorus pesticides in milk and butterfat, it was found that the recoveries of diazinon from butterfat, calculated relative to organic solutions of standard compounds, were 125% and 84% for splitless and hot on-column injections, respectively (Erney et al. 1993). Recoveries from milk were not dependent on the mode of injection. It was concluded that the sample matrix served to increase diazinon transfer to the GC column by reducing thermal stress imposed on the analytes and by blocking active sites within the injector. Therefore, on-column injection should be used in order to prevent bias when organic solutions of standard compounds are used for quantitation; if this is not possible, the matrix must be present at low concentrations or the calibration standards must be prepared in residue-free samples to avoid unknown bias.

6.2 ENVIRONMENTAL SAMPLES

Diazinon residues are found throughout the environment in air, water, soil, sediments, sludges, and other solid wastes because of the use of this compound for agricultural purposes. The use of diazinon on crops presents the possibility of residues in products for human consumption, making food an important potential route of exposure for this compound.

Diazinon can be measured in air after pre-concentration from air onto some adsorbent material with subsequent extraction. Following extraction from the adsorbent, separation and detection methods include GUMS (Hsu et al. 1988; Kuwata and Yasuhara 1994) GC/NPD (Williams et al. 1987), and GC/FPD in the P mode (NIOSH 1994). The method of Williams et al. (1987) applicable to both diazinon and diazoxon. The NIOSH method (Method 5600, NIOSH 1994) has been fully validated for use in occupational settings where regulatory exposure limits are of concern.

Many methods for the determination of diazinon in environmental media have been published by the EPA (see Table 6-2). For surface water and industrial and municipal waste waters, Methods 622, 614, and 1657 and preparation Methods 3510/3520 in conjunction with analytical Method 8140 (EPA 1986a, 1986b, 1986c, 1992a, 1992b, 1992c) can be used. All of the methods employ some form of liquid/liquid extraction, extract volume reduction, and GC in conjunction with selective detection

(e.g., FPD, thermionic detection, or MS). Reported LODs range from a high of approximately 6 μg/L (SW846 Method 8140 applied to water) down to 12 ng/L (Method 614) (EPA 1986a, 1992b). In most cases, the recovery will be dependent upon the particular matrix. For Method 1657, recoveries in the range of 60-120% are considered acceptable. Average recoveries were reported to be 67% for Method 622 and 94% for Method 614 (EPA 1992a, 1992b, 1992~). Methods are also available for soils, sludges, sediments, and solid wastes. Sample preparation typically involves liquid/liquid extraction in a separatory funnel, in a Soxhlet extractor, or with sonication. The more complex samples (some waters and most soils, sediments, sludges, or solid wastes) need to be subjected to some clean-up method before analysis. The use of Florisil, GPC, and SPE are common approaches. Diazinon is determined by GC/FPD (EPA 1986a, 1992c).

Although not specific for diazinon, some general interferences were noted in the EPA methods. Careful attention must be paid to the cleanliness of the reagents and glassware (EPA 1986b, 19868). Trace impurities can become major impurities during extract concentration steps. In addition, soap residues on glassware can cause the degradation of organophosphorus pesticides (EPA 1986b).

Many other methods were reported for the determination of diazinon in water. Sample preparation methods include either some form of liquid/liquid extraction or the use of SPE, usually C_{18}-silica, for isolation of diazinon residues. Mallet et al. (1990) reported a method for environmental water based on high performance liquid chromatography/ultra violet (HPLCKJV) absorbance detection with either direct injection of the water or of an aliquot of an extract. The LODs were as low as 0.5 μg/L with the extraction approach. Mattern et al. (1991) reported a LOD for diazinon in surface water of 0.0005 ppb using GC in conjunction with chemical ionization ion trap MS. Lopez-Avila et al. (1985) reported an isotope dilution GC/MS selected ion monitoring (SIM) method that is applicable to water or soil after solvent extraction. Recoveries were stated to be 89% at 1 ppb in water and 103% at 20 ppb in soil. An LOD of 0.025 μg/kg was reported for diazinon in water with a recovery of 92% (2% RSD) by Seiber et al. (1990). SPE provides an easy method to isolate residues and can greatly reduce the amounts of solvent used in sample preparation. Driss et al. (1993) preconcentrated diazinon from drinking water onto C_{I8}-silica or polystyrene-divinylbenzene co-polymer with a subsequent backflush onto an HPLC column (UV detection). LODs as low as 30 μg/L were reported. Kwakman et al. (1992) preconcentrated diazinon from drinking and river water onto C_{18}-SPE disks and eluted the adsorbed compounds directly into a GC pre-column. The solvent was vented away from the analytical column during the elution step. Detection was by NPD and excellent LODs (20 pg/L) and

recoveries (greater than 95% with less than 4% RSD at 200 pg/L) were reported. Although most of the SPE methods boasted good recoveries and LODs, one reference noted that the pesticide can associate with dissolved organic matter (primarily humic materials) resulting in poor retention by the SPE material (Johnson et al. 1991). This can reduce method recoveries.

Diazinon has a finite vapor pressure (see Chapter 3) and thus will be present in the air. A method for diazinon in air has been reported that is based on the use of polyurethane foam (PUF) to adsorb the pesticide from the air as the air is pulled through the PUF (Hsu et al. 1988). The PUF is then Soxhlet-extracted and the extract volume reduced prior to capillary GC/MS analysis. An LOD of 55 ng/m^3 (5.5 m^3 sample) and recovery of 73% were reported. Another study was described in which the diazinon levels in indoor air were monitored following periodic application of the pesticide for insect control (Williams et al. 1987). In this method, air is pulled through a commercially available adsorbent tube to concentrate diazinon. The tube is then extracted with acetone prior to GC/NPD analysis. No data were provided for the LOD, but recoveries in excess of 90% were reported at the 0.1 and 1 μg/m^3 levels. This paper also indicated that diazinon can be converted to diazoxon by ozone and NO_x in the air during the sampling process.

SFE also would appear to have utility in sample preparation methods. Lopez-Avila et al. (1992) applied SFE to the recovery of a variety of analytes, including organophosphorus pesticides, from solid matrices. The unoptimized extraction from sand gave a recovery of 54% for diazinon. Supercritical trifluoromethane has been shown to extract diazinon from glass beads with a recovery of 86% (Hillmann and Bachmann 1995). Organophosphorus pesticides have also been recovered from Tenax-GC, an adsorbent used to collect diazinon during air sampling, and analyzed directly by GC (Raymer and Velez 1991). More SFE-based methods will likely appear in the future. Supercritical fluid chromatography (SFC) has also been used for the determination of diazinon in water where 75 μL were injected (Zegers et al. 1994b). Using thermionic detection, the LOD was about 1 μg/L (1 ppb) with a reproducibility of better than 7% at the 5-15 μg/L level. The same authors also published an SFC-based method for cucumber, lettuce, and grapes (Zegers et al. 1994a) but did not specify the LOD and recovery.

The determination of diazinon in foods is important because this chemical is used as a pesticide on plant crops and, at least in some cases, in pesticide dips for the control of parasitic infestations in animals (Brown et al. 1987; Miyahara et al. 1992). Because animals are exposed to this compound,

both via pesticide dips and by ingestion of crops to which diazinon has been applied, some methods have been reported for animal products. The majority of methods, however, deal with the determination of residues in plant products. Most of the analytical methods found that describe the extraction from, and determination of, diazinon residues in various crops (plant materials) were developed as part of multiresidue methods. They are based on homogenization of the sample with an organic solvent (polar or non-polar); the isolation of the residues from this initial extract; and, usually, some additional cleanup prior to the analysis of the extract by GC. The most common non-MS modes of detection exploit the presence of phosphorus or sulfur (FPD) or phosphorus or nitrogen-thermionic, NPD. Whenever possible, the MS mode of detection also provides confirmation of the structure thus increasing the certainty of the identification. The acquisition of full-scan data is the most convincing for confirmatory analyses, although the method LOD tends to be adversely affected. The use of SIM MS can improve the LOD over full-scan analysis and can often provide sufficient selectivity, if the appropriate number of specific ions are chosen, for high confidence in the chemical identity. It is also common to see the analysis of a particular extract on two GC columns coated with phases of different selectivity. The coelution of a peak in the sample with the peak associated with a chemical standard on both stationary phases greatly increases the probability that the unknown is indeed the same chemical as the standard.

Three standardized methods were found in the *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC 1990). The first of these methods is based on the extraction of crops (kale, endive, carrots, lettuce, apples, potatoes, and strawberries) with ethyl acetate and isolation of the residue followed by a sweep codistillation cleanup prior to GC/thermionic detection (Method 968.24). The second of these methods utilizes Florisil column chromatography clean-up followed by GUFPD (Method 970.53). In the third method (Method 970.52), the sample is extracted with acetonitrile, and the residue is partitioned into petroleum ether followed by Florisil clean-up and GC/KCl thermionic detection. Identifications are based on combinations of gas, thin-layer, and paper chromatography. The recovery for diazinon in this method is stated to be greater than 80%; no data on limits of detection were given.

Several methods employ the homogenization of the plant material with aqueous acetonitrile (Hsu et al. 1991; Liao et al. 1991) or other polar organic solvents such as acetone/methanol mixtures (Hong et al. 1993). Phase separation is brought about with the addition of a salt. The acetonitrile approach is preferred by the California Department of Food and Agriculture because of the higher recoveries

possible (see Table 6-2) (Lee et al. 1991). The advantage of acetonitrile is found in its ability to more readily solvate residues and in the ease with which the phase separation can be accomplished through the addition of salt (Lee et al. 1991). Reported LODs for diazinon were typically l0-50 ppb. One of the methods eliminated any clean-up steps after the initial extraction (Hsu et al. 1991) to provide a method with a faster turnaround time with some loss in sensitivity (LOD approximately 100 ppb) relative to the purified samples.

The method published by Kadenczki et al. (1992) combined sample extraction with extract cleanup by adsorbing a homogenized sample (various fruits and vegetables) onto the surface of activated Florisil to obtain a free-flowing powder. This was packed into a column and the organophosphate residues were eluted with ethyl acetate or methylene chloride. Good recoveries (91-103%) were obtained at the 0.05 mg/kg (50 ppb) level, with an LOD estimated to be 4 μg/kg (4 ppb) when using GUNPD.

Methods found for the determination of diazinon in animal products also used homogenization with a polar organic solvent as the first step in residue recovery. Toyoda et al. (1990) isolated diazinon from milk via partition into methylene chloride after extraction of the milk with 70% acetonitrile in water. Based on GC/FPD, an LOD of 10 ppb and a recovery of 89% (3.8% relative standard deviation) at 100 ppb were reported. Diazinon residues in eggs were studied (Leoni et al. 1992) after blending the eggs with acetone and partitioning into dichloromethane and acetone followed by C_{18}-silica SPE. Based on GC/FPD analysis, an LOD of 1 ppb and a recovery of 93% at 13 ppb were reported.

Some alternate GC detection schemes that can provide unique selectivity in the determination of organophosphorus pesticides were reported. Although they require more expensive hardware, such approaches might prove useful in selected applications or if the hardware is currently available in the laboratory. Stan and Kelner (1989) have described a method for the GUMS confirmation of organophosphorus pesticides using pulsed positive-negative ion chemical ionization (PPNICI). This method generates base peaks with high masses in the positive ion mode and group-specific fragments of high intensity-in the negative ion mode. Ions to be monitored are recommended for 72 compounds, including diazinon.

With GC in conjunction with atomic emission detection (AED) and the simultaneous monitoring of emission wavelengths from a microwave plasma for several heteroatoms (e.g., S, P, Cl, N), selectivity in the analysis of complex samples can be greatly increased (Wylie and Oguchi 1990). GUAED was

claimed to provide greater selectivity than the more common GC detectors (FPD, NPD) used for the analysis of organophosphorus compounds (Lee and Wylie 1991). Methods for diazinon in water using GUAED have been published (Eisert et al. 1994; Hankemeir et al. 1995). The products formed in the plasma can also be introduced into a mass spectrometer to increase selectivity and provide additional information about the atomic composition (Story and Caruso 1993). Both AED and NICI should be applicable to both biological and environmental samples.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of diazinon is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of diazinon.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Section 2.6.1 reported on biomarkers used to identify or quantify exposure to diazinon. Some methods for the detection of the parent compound in biological samples were described above. The parent chemical is quickly metabolized so the determination of metabolites can also serve as biomarkers of exposure. The most specific biomarkers will be those metabolites related to 2-isopropyl-6-methyl-4-hydroxypyrimidine. A method for this compound and 2-(1‘-hydroxy- 1‘-methyl)-ethyl-6-methyl-4-hydroxypyrimidine in dog urine has been described by Lawrence and Iverson (1975) with reported sensitivities in the sub-ppm range. Other metabolites most commonly detected are 0,0-diethylphosphate and 0,0-diethylphosphorothioate, although these compounds are not specific for diazinon as they also arise from other

diethylphosphates and phosphorothioates (Drevenkar et al. 1993; Kudzin et al. 1991; Mount 1984; Reid and Watts 1981; Vasilic et al. 1993). Another less specific marker of exposure is erythrocyte acetyl cholinesterase, an enzyme inhibited by insecticidal organophosphorus compounds (see Chapter 2). Methods for the diazinon-specific hydroxypyrimidines should be updated and validated for human samples. Rapid, simple, and specific methods should be sought to make assays readily available to the clinician. Studies that relate the exposure concentration of diazinon to the concentrations of these specific biomarkers in blood or urine would provide a basis for the interpretation of such biomarker data.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Human exposure to diazinon occurs via inhalation of ambient air; ingestion of contaminated food and water; and dermal uptake through occupational and nonoccupational contact with contaminated soils, surface water, and commercial preparations. Methods have been reported for the measurement of diazinon in various foods, soils, sludges, sediment, solid wastes, waste water, drinking water, and air. The MRLs established for diazinon are 0.009 mg/m^3 (90 μg/m^3; 3.7 ppb) for intermediate-duration inhalation and 0.0002 mg/kg/day for intermediate duration oral exposure. The methods of Hsu et al. (1988) (LOD of 55 μg/m^3) and Kawata and Yasuhara (1994) (LOD of 0.5 μg/m') are adequate for the determination of diazinon in air. If a 70-kg individual is assumed, method LODs of 0.007 mg/L (7 ppb) and 0.007 mg/kg (7 ppb) in water and foods, respectively, are required for the method to be adequate at the oral intermediate MRL. All of the methods for detection of diazinon in water shown in Table 6-2 are adequate. With regard to foods, the methods of Kadenczki et al. (1991) and Leoni et al. (1992) for detection of diazinon are adequate. Methods for other non-fatty crops would need to be validated or developed if routine use were desired. Additional methods for detection of diazinon in fatty foods are needed to permit the evaluation of the residues in those fatty media.

There are also methods for the analysis of diazinon degradation products in air, water, and soil. Williams et al. (l987) published a method for diazinon and its oxon (diazoxon) in air. Other methods have been reported for diazinon, its oxon, and hydrolysis products in water (Suffet et al. 1967), soils and water (Lichenstein et al. 1968), and soil (Burkhard and Guth 1979). The hydrolysis product 2-isopropyl-6-methyl-4-hydroxypyrimidine was studied along with diazoxon in submerged soil (Sethunathan and Yoshida 1969). Suffet et al. (1967) demonstrated the ability of GC to separate diazinon, diazoxon, and 2-isopropyl-6-methyl-4-hydroxypyrimidine. However, no validated methods

for the determination of diazoxon or 2-isopropyl-6-methyl-4-hydroxypyrimidine were found. Thus, additional methods are needed for the quantitative analysis of diazinon transformation products in environmental matrices. It will also be important to establish MRLs for the transformation products to put the analytical requirements into perspective.

6.3.2 Ongoing Studies

The following ongoing studies on analytical methods for diazinon were found.

1) The University of Maryland, Eastern Shore, Human Ecology, is evaluating the ability of various fabrics to reduce exposure to diazinon and is evaluating the effectiveness of decontamination procedures.

2) The Department of Food Science at the University of Maine, Orono, is developing methods for diazinon in food, water, and soils based on GC/AED, HPLC, and immunoassays.

3) The University of Nevada, Range Wildlife and Forestry, Reno, is developing and evaluating methods for determining diazinon and conversion products in air and atmospheric moisture.